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Research Article

MICROSCOPIC STUDIES OF LEAF AND ANTIOXIDANT ACTIVITY OF AERIAL PARTS OF FIELD BINDWEED

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A B S T R A C T

Herbal medicine has been commonly used over the years for treatment and prevention of diseases and health promotion. *Convolvulus arvensis* is a field weed with many therapeutic properties. Usually, the weeds are considered to be useless and of no therapeutic importance. But Bindeed has potential to arrest the disorder of health and possess promising therapeutic value. This research includes the anti oxidant activity of this plant using DPPH and Hydrogen peroxide assay and the authentication of the plant is done by performing the microscopic studies.

INTRODUCTION

Antioxidants

Increase in the level of oxidative stress or production in large number of free radicals are the main cause of many dangerous diseases in humans like myocardial infarction, cancer, diabetes etc[1]. At low concentrations, free radicals have a vital role in physiological regulations and cellular signaling processes but when the concentrations of free radical increases in the body it causes damage to various cell components like proteins, lipids and DNA. In DNA oxidative stress can cause base damage as well as strand breaks in DNA[2].

Antioxidants are the substances which have the property of counteracting the effect of highly reactive and harmful free radical formed due to oxidation reactions. The main role of anti oxidants is to delay or inhibit oxidation of substrate[3]. Natural antioxidants are more preferred now days as compared to synthetic antioxidants due to presence of toxic and carcinogenic substances in them where as natural antioxidants are safer to use and non-toxic.

Convolvulus arvensis

Convolvulus arvensis belongs to family Convolvulaceae. The plants under this family have characteristic climbing property, are mainly herbs or shrubs and flowers have a cymose type of inflorescence.

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The leaves of *Convolvulus arvensis* may be broader or narrower based on the variety but basic characters are ovate oblong to lanceolate type and have cordate or saggitate base. The stems of this plant have trailing or twining property and are basically hairless but can be pubescent or glabrous. The petals of the flower are pink or white in colour and the Inflorescence is axillary cyme composed of one or three flowers. The flower is bisexual, five stamens of unequal lengths are attached to the base of the corolla and pistil is compound with two thread-like stigmas. The fruits of this plant are capsules and 4-valved[4,5]. Phytochemical studies show this plant contains alkaloids like Calystegins[6], Lipids like Iso-butyric acid, Palmitic Acid, Oleic Acid, Linolenic acid etc[7], Flavanoids like 7-o-rutinoside, Kaempferol -3-o-β-D-Glucoside etc[8], Phenolic acid like Caffeic acid, p-coumaric acid, Vanillic acid etc[9], steroids like Stigmasterol, β sitosterol etc[10] and many others. Traditionally, this plant was used in treating skin ulcers, reducing inflammation and swelling of wounds[11]. It was also used as anti-spasmodic and anti-haemorrhagic as well as anti-angiogenic effect[12]. The aerials parts was also used as laxative[13] and in treating dandruff[14]. It was also used in treating asthma[15]and jaundice[16]. Recent studies shows that *Convolvulus arvensis* have many pharmacological uses like Cytotoxic activity[17], Immunostimulant activity[18], Vasodilating effect[19], Hepatoprotective effect[20], Diuretic effect[21] and much more.

MATERIALS AND METHODS

Collection and preparation of plant extract

The leaves of *Convolvulus arvensis* were collected from local nursery, Amritsar, Punjab in the month of July and August and

was authenticated. The plant was dried in shade for 4-5 days with occasional drying in sunlight.

Extraction of plants

The extraction was carried out using Soxhlet apparatus using chloroform and methanol as solvents. The powdered plant was added to the system and by using methanol the process of extraction was carried out at 60-70 ^{0}C. After the extraction, the solvent containing the extracts was collected and the plant residue were dried overnight and then again Soxhlet apparatus was set using dried marc with chloroform as solvent. The whole process took 3-4 days to complete. Later the extracts were concentrated by removing solvent and kept in dessicator till further use. Afterwards, the extracts were tested for anti oxidant activity of the plant by performing DPPH (2,2-dipheny 1-picryl hyrazyl) and Hydrogen peroxide assays.

DPPH Protocol

DPPH 0.002 mg was weighed and added in volumetric flask and volume was made upto 100ml with methanol. Ascorbic acid 20 mg was weighed and mixed with 20 ml of methanol. Test extracts (methanol and chloroform) were weighed 0.002 mg and mixed with 20 ml of methanol. Serial dilutions 50,100,150 µg/ml of standard i.e. ascorbic acid and test extracts were prepared by taking 50, 100,150 µg of standard and test extracts respectively and mixed with 20 ml of methanol. 2ml of above solutions i.e. from the standard and test extracts were taken and 2 ml of DPPH were added to all of them. The test tubes were incubated in dark for 30 minutes. Methanol was used as blank and absorbance was noted at 514 nm [22,23].

Hydrogen Peroxide Assay Protocol

The solution of hydrogen peroxide was prepared by dissolving 0.136 gm of hydrogen peroxide in 100 ml of phosphate buffer, which is used as standard. 5 mg of test extracts i.e. chloroform and methanol were dissolved in 5ml of distilled water with 3 ml of hydrogen peroxide solution. Phosphate buffer solution without hydrogen peroxide was used as blank. Serial dilutions i.e. 50,100,150 µg of standard and test were prepared respectively and their absorbance was taken at UV spectrometer at 230 nm which was determined 10 minutes later against the blank solution. A graph between the concentration and their respective absorbance was checked [24].

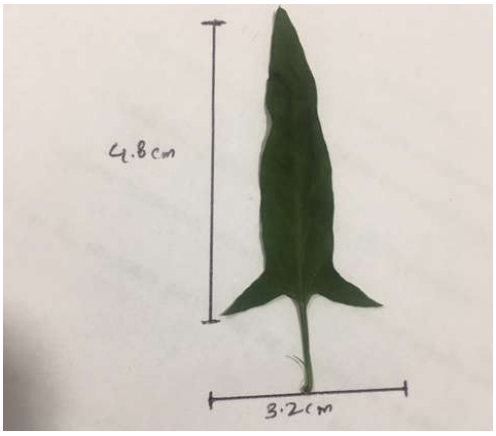
Microscopy of leaves of Convolvulus arvensis

For the microscopy of the leaves of *Convolvulus arvensis* different stains were used to check the presence of different types of cells. After the collection of leaves, they were washed 2-3 times with water and then treated with chloral hydrate for 10-15 minutes to remove chlorophyll for the better visibility of structures in the leaves. The T.S. of leaves was first observed under Compound microscope and then under Olympus microscope and microscopic characters were observed.

RESULTS AND DISCUSSIONS

Macroscopy

Convolvulaceae is a family of trailing and twinning plan and is recognized by funnel shaped flowers and radially symmetrical corolla[25]. The average length of the leaf was found to be 4.8 cm and width is around 3.2 cm bearing dark green colour, long petiole is present, apex is acute and base is winged giving rise to cordate shape.



Microscopy of Leaves

The TS of leaf of *Convolvulus arvensis* revealed the presence of different microscopic characters like stomata, epidermis palisade cells, spongy parenchyma, vascular bundles etc.

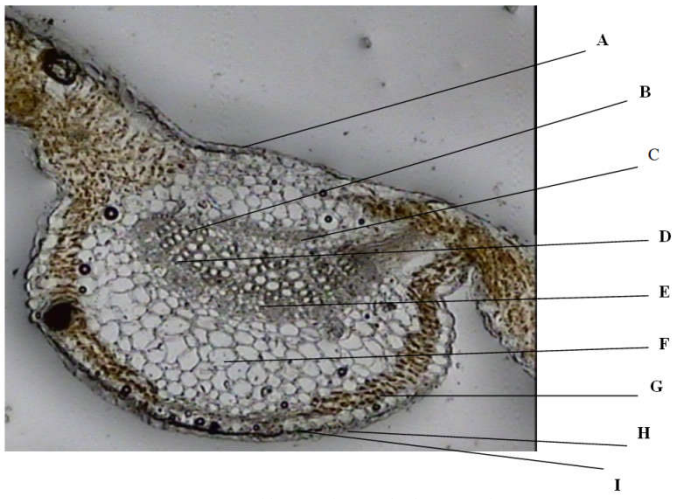


Fig 1 T.S. of leaves of Convolvulus arvensis

Fig 1 A = Upper epidermis, B = Xylem, C = Schlernchyma sheath, D = Phloem, E = Schlernchyma sheath, F = Parenchyma, G = Lower palisade containing chlorophyll, H = Lower epidermis, I = Cuticle

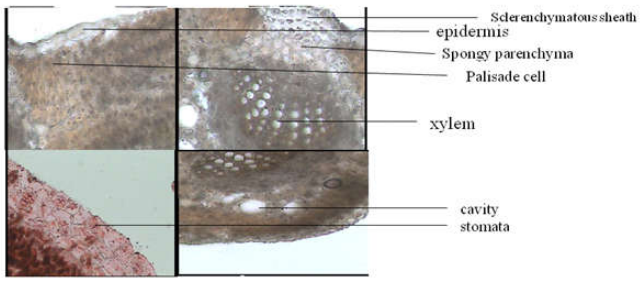


Fig 2 Microscopic characters of *Convolvulus arvensis* leaf

DPPH Assay

Concentration (µg)	Absorbance (nm) Ascorbic acid		Mean	Absorbance Methanol		Mean	Absorbance (nm) Chloroform		Mean
50	0.399	0.369	0.384	0.341	0.285	0.299	0.312	0.289	0.300
100	0.585	0.547	0.566	0.499	0.435	0.467	0.507	0.486	0.496
150	0.899	0.789	0.844	0.59	0.612	0.601	0.70	0.672	0.686

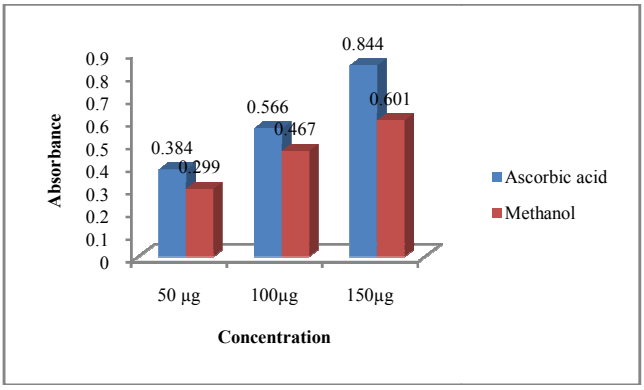


Fig 3 graphical representation of DPPH assay for methanolic extract of *C. arvensis*

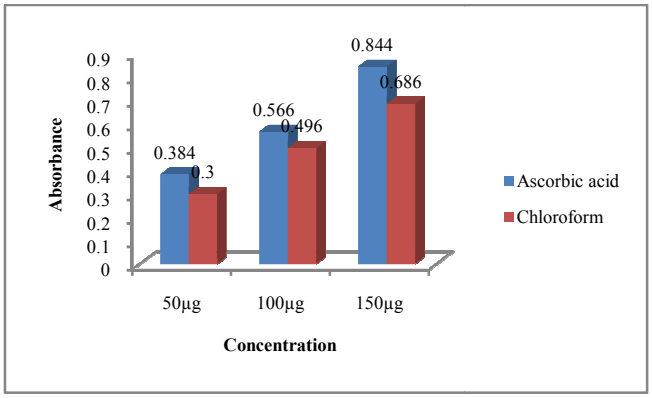


Fig 4 graphical representation of DPPH assay for chloroform extract of *C. arvensis*

Table Absorbance of different extracts of *C. arvensis* by H_2O_2 Assay

Concentration (µg)	Absorbance (nm) Hydrogen peroxide		Mean	Absorbance Methanol		Mean	Absorbance Chloroform		Mean
50	0.138	0.115	0.126	0.102	0.088	0.095	0.104	0.094	0.099
100	0.322	0.310	0.316	0.275	0.247	0.261	0.203	0.186	0.194
150	0.54	0.524	0.532	0.389	0.358	0.373	0.36	0.314	0.337

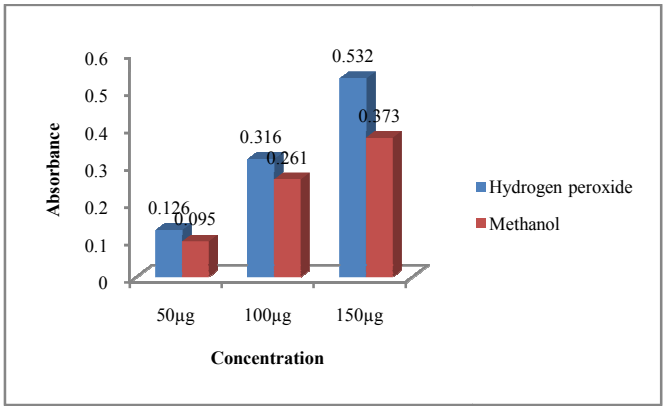


Fig 5 Graphical representation of H_2O_2 Assay for methanolic extract of *C. arvensis*

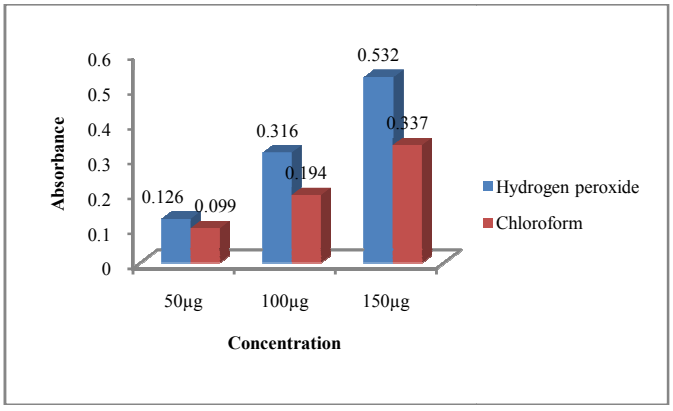


Fig 6 Graphical representation of H_2O_2 Assay for chloroform extract of *C. arvensis*

CONCLUSION

The anti oxidant potential of aerial parts of *Convolvulus arvensis Linn* in different extracts i.e. Methanol and Chloroform at different concentrations is found to be dose dependent. In DPPH and Hydrogen peroxide assay the results are more comaparble to standard in chloroform extract.

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